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Changes in Prolactin Cell Activity in the Mudskipper, *Periophthalmus chrysospilos*, in Response to Hypotonic Environment

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ABSTRACT—Changes in plasma electrolyte concentrations and prolactin and growth hormone cell activities were examined in the euryhaline mudskipper, *Periophthalmus chrysospilos,* after exposure to hypotonic environment. When the fish were fully submerged in fresh water, they died within 2 days. No mortality was seen either in 15% seawater or in fresh water, however, when they had the liberty to be in or out of the water, although plasma sodium and calcium concentrations of the fish kept in a freshwater aquarium for 7 days were significantly lower than those in 100% or 15% seawater. There was no difference in plasma sodium levels between the fish in 100% seawater and those in 15% seawater. Prolactin- and growth hormone-secreting cells in the pituitary were identified by immunocytochemical staining using antisera raised against the salmon hormones. Significant increases in the nuclear and cell sizes of prolactin cells were observed in the fish in fresh water as compared with those in 100% or 15% seawater, whereas no change was seen in growth hormone cells. The activation of prolactin cells supports its important roles in freshwater osmoregulation.

INTRODUCTION

Mudskippers are amphibious and euryhaline gobiid teleosts, mostly belonging to the genera *Periophthalmus* and *Boleophthalmus.* They are widly distributed in the intertidal environments of the Indian and West Pacific Oceans and along the coasts of tropical West Africa, where the water salinity varies significantly during rainy season or extreme hot weather. They spend th egreater part of their lives out of water, moving about over the surfaces of shores some distance from the tide line. Thus, they provide a unique model for studies on both terrestrial adaptation and aquatic osmoregulation.

Although several hormones have been implicated in teleost osmoregulation, prolactin's roles in freshwater adaptation seem to be best defined [1–3]. Recently, there is an increasing body of evidence indicating an osmoregulatory role of growth hormone, particularly during seawater adaptation of salmonid fish [3–6]. Although osmoregulatory processes of the mudskippers, especially of Periophthalmidae, have been the subjects of several physiological and behavioral studies [7–11], little has been studied on their hormonal control, except for a report by Lee and Ip [12] on environmental salinity and plasma prolactin and thyroid hormone levels. The presnet study was undertaken to clarify the role of prolactin and growth hormone in maintenance of hydromineral balance of the mudskipper, *P. chrysospilos*.

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MATERIALS AND METHODS

Periophthalmus chrysospilos, weighing about 2 g, were collected along the shore near the Pasir Ris estuary at the East Coast of Singapore. They were shipped to Ocean Research Institute of University of Tokyo, and kept in aquaria containing 50% (salinity 17 ppt) seawater at 25)°C. Rocks were provided for the fish to climb on. They were fed tubifex (*Chironomus* larvae). No attempt was made to separate the sexes.

After acclimation to the laboratory condition for more than 2 weeks, some fish were fully submerged either in 100% seawater (Na, Ca, Mg; 450, 10, 50, respectively in mM) or in fresh water (Na, 0.4; Ca, 0.4; Mg, 0.1) by confining them in a net. Since most of them died in fresh water within 2 days, in the next experiment, they were transferred to 100% seawater, 15% seawater (Na, 68; Ca, 1.5; Mg, 7.5) or fresh water with the liberty to be in or out of water. They were sacrificed after 1 week. The caudal peduncle was severed and the blood was collected from the caudal vessels into capillary tubes. The tubes were centrifuged at 5,000×*g* for 5 min. The plasma sodium, calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Hitachi 180–50).

Immunocytochemical staining was carried out according to the peroxidase-antiperoxidase (PAP) method as described by Naito *et al.* [13]. Alternate sagittal sections of the pituitary were stained with either anti-chum salmon prolactin antiserum [13] or anti-chum salmon growth hormone antiserum [14]. In each animal, tissue sections which seem to include the largest number of immunostained cells were chosen to measure cross sectional cell and nuclear areas for both prolactin and growth hormone cells. They were projected on a TV screen connected to a high contrast TV camera (Hamamatsu Photonics, C1965), and those cells which had observable nuclei were selected for outlining their boundaries and nuclei on translucent paper. The areas of the cells and the nuclei were then determined by use of a tablet digitizer and a computer.

Effects of changes in environmental salinity on plasma electrolyte concentrations and on cell and nuclear sizes were statistically analyzed by the Duncan's new multiple range test or the Kruskal-Wallis test following the Bartlett's test using computer programs written by Prof. Susumu Ishii of Waseda University. The Bartlett's test was applied to examine whether there was a difference in variances of test groups. In case of no significant difference, the Duncan's test was applied to compare the means. When there was a significant difference ($P<0.05$) among the variances, the data were analyzed by the Kruskal-Wallis test.

RESULTS

When undisturbed, the mudskippers in aquaria were found virtually all of the time out of water, frequently resting on aquarium wall or on a rock with the foreparts out of the water and tail submerged. When 6 fish were fully submerged in fresh water, 4 fish died within 48 hr. The plasma sodium concentrations of the remaining, nearly moribund, fish were 87 and 92 mM, whereas those of the fish submerged in 100% seawater for 48 hr were 150±3.8 mM (n=3).

When they were transferred from 50% seawater to aquaria containing 100% seawater, 15% seawater or fresh water with the liberty to be in or out of

Table 1. Effects of environmental salinity on plasma electrolyte concentrations of the mudskipper, *Periophthalmus chrysospilos*

Environment*	No. of fish	Electrolyte concentrations (mM)**		
		sodium	calcium	magnesium
100% seawater	7	159±2.5	3.1±0.07	1.4±0.14
50% seawater	7	159±2.0	3.0±0.20	—
15% seawater	5	158±3.1	3.0±0.26	1.0±0.04†
fresh water	5	110±5.5†	2.3±0.10†	0.9±0.11†

* Fish were acclimated to 50% seawater and transferred to each environment for 7 days, with the liberty to be in or out of the water. ** Mean±SEM.

† Significantly ($P<0.01$) different from the value in the fish in 100% seawater by the Duncan's new multiple range test.

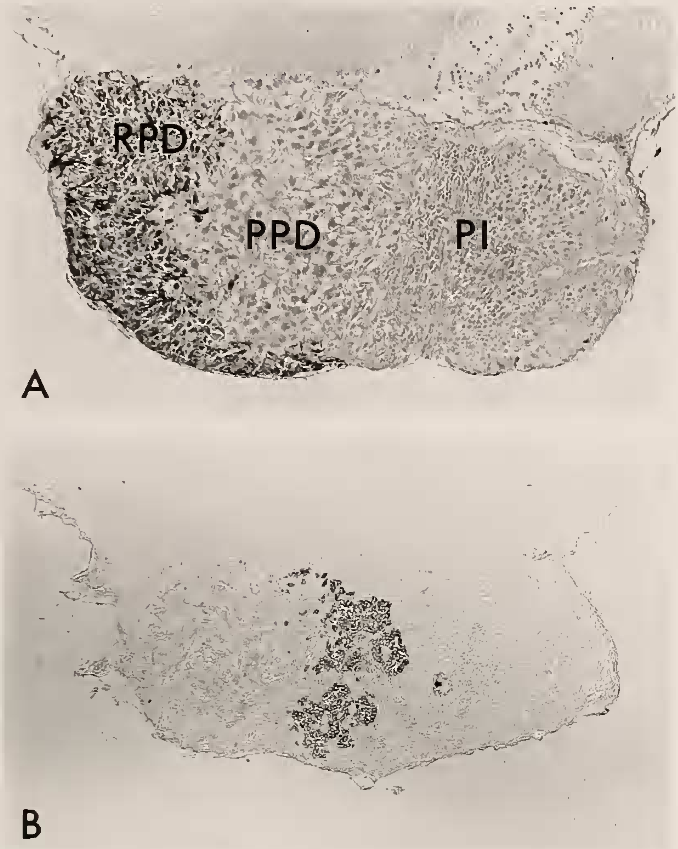

FIG. 1. Sagittal sections of a mudskipper pituitary stained with an anti-chum salmon prolactin rabbit serum (A) and with anti-chum salmon growth hormone rabbit serum (B). PI, pars intermedia; PPD, proximal pars distalis; RPD, rostral pars distalis. ×136.

the water, no mortality was seen in any environment for 7 days. However, the plasma sodium and calcium levels in the fish kept in a freshwater aquarium for 7 days were significantly lower than in those in 100% or 15% seawater aquaria. There was no difference in plasma sodium and calcium levels between the fish in 100% seawater and those in 15% seawater. Plasma magnesium concentrations of the fish in 15% seawater and in fresh water were significantly lower than that of the fish in 100% seawater (Table 1).

Pituitary of the mudskipper was partly embedded in the basal part of the hypothalamus. Prolactin- and growth hormone-secreting cells in the pituitary were identified by immunocytochemical staining using antisera raised against the salmon hormones (Fig. 1). Prolactin cells occupied most part of the rostral pars distalis. The cells were trapezoid or irregular in outline, with a round nucleus situated mostly in the center of the cell. Growth hormone cells were found exclusively in the proximal pars distalis. The cells and nuclei of growth hormone cells were irregular in shape, and the nucleus was located frequently near the periphery of the cell.

The sizes of cells and nuclei of prolactin cells were significantly ($P<0.01$) grater in the fish in fresh water than those in 100% or 15% seawater, whereas no change was seen in growth hormone cells (Figs. 2, 3).

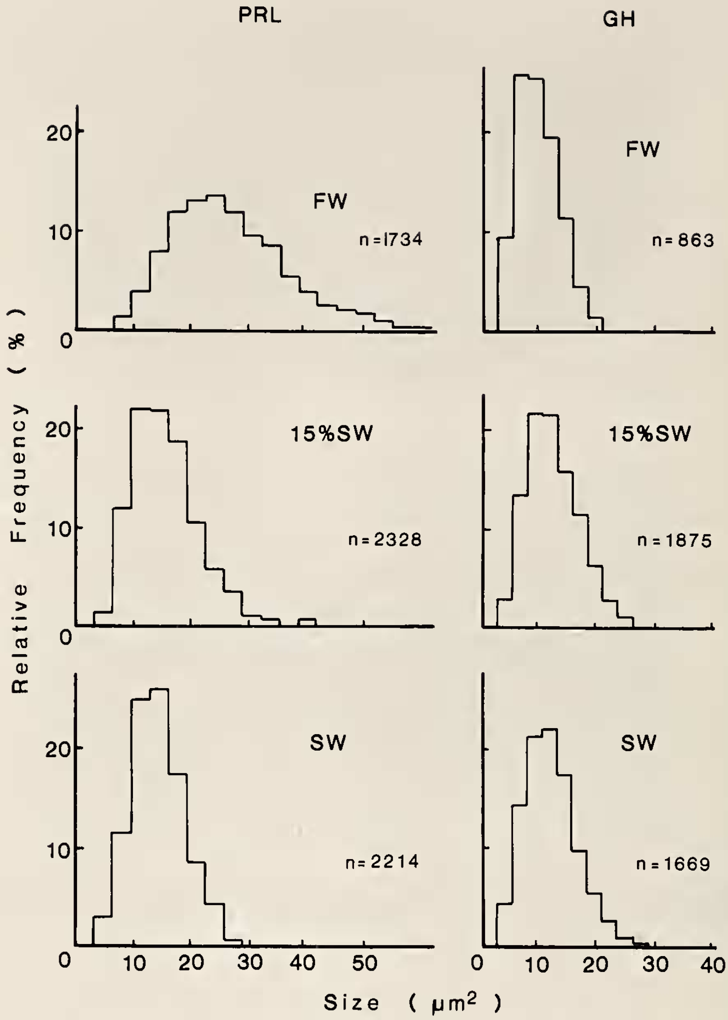

FIG. 2. Frequency histograms of cell sizes of prolactin (PRL)- and growth hormone (GH)-cells of the mudskipper exposed to different salinities. The fish were kept in aquaria containing fresh water (FW, n=3), 15% seawater (15% SW, n=4) or 100% seawater (SW, n=5) with the liberty to be in or out of water. Cross sectional cell area was measured using a sagittal section which included the largest number of immunostained cells in each fish. The number of prolactin cells as well as growth hormone cells ranged from 300 to 750 per section. All the values were combined for each group, and expressed as relative frequency. Total number of the cells measured (n) is indicated in each histogram. The mean cell size of prolactin cells of the fish in fresh water was significantly ($P < 0.01$) different from those of the fish in 15% SW or in SW by the Duncan's new multiple range test.

DISCUSSION

In Singapore, *Periophthalmus chrysospilos* lives in the littoral zone of the shore, where the water salinity vaires between 30–34 ppt. They usually lie on land close to the water edge or clamber onto rocks and mangrove roots. When disturbed, they would skim across the water surface towards the sea in several jumps. Their physical mobility is great enough so that it is unlikely that they are trapped in evaporating tide pools. The only serious salinity stress they are likely to encounter is that of low salinity during the rainy season or by dilution of the water by the river during low tide,

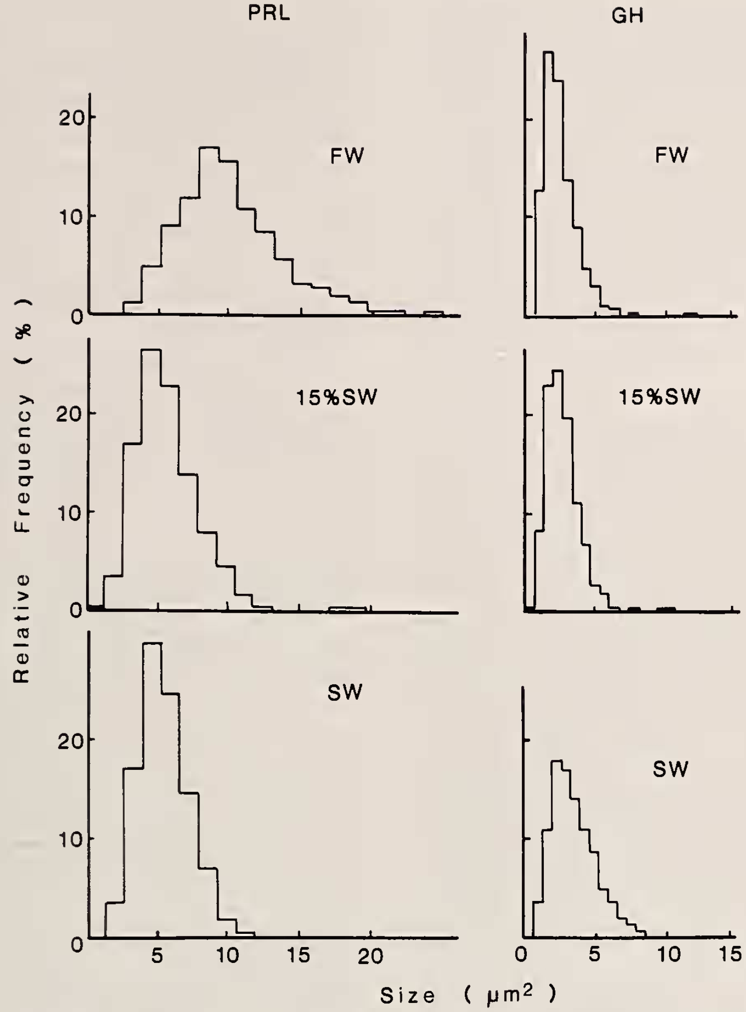

FIG. 3. Frequency histograms of nuclear sizes of prolactin (PRL)- and growth hormone (GH)-cells of the mudskipper exposed to different salinities. The mean nuclear size of the prolactin cells of the fish in fresh water was significantly ($P<0.01$) different from those of the fish in 15% SW or in SW by the Kruskal-Wallis test. See also legend to Fig. 2.

although low salinities would not persist for more than a few hours. As shown in this study, *P. chrysospilos* was surprisingly euryhaline, especially when they were allowed to move into or out of water *ad libitum*. They adjusted their plasma electrolyte levels at constant levels in hypertonic 100% or 50% seawater or hypotonic 15% seawater; a significant reduction in the plasma sodium level was seen when they were kept in a freshwater aquarium.

When they were fully submerged in fresh water, they were unable to survive for more than 48 hr. This is in agreement with previous observation of this species by Lee *et al.* [11] and Lee and Ip [12], indicating that they were unable to survive in deionized water for more than 18 hr. According to Gordon *et al.* [7], *P. sobrinus* from Madagascar tolerated direct transfer from 100% seawater to salinities as low as 20% seawater, even though they were forced to remain continually in water. Direct transfer to fresh water, however, caused death within 1–3 days, although they survived in fresh water when they had previously been adpted to 20% for 6 days. Gordon *et al.* [10] also reported that *P. cantonensis* showed no preference for any particular salinity, but avoided exposure to fresh water. It is not clear why the mudskipper submerged in fresh water failed to adjust their plasma electrolytes, whereas the fish with the liberty to be in or out of water had no difficulty. It is unlikely that they need to gulp air for respiration, since *P. chrysospilos* and *P. cantonensis* survived in aerated seawater without access to air for more than 5 days [11, 12]. One of the reasons why the fish submerged in fresh water failed to osmoregulate would be a severe hydration resulting from the fish being forced to remain continuously in fresh water.

The pituitary of the gobiid fish is unique among other teleost species, in that the whole adenohypophysis is nearly buried into the hypothalamus and that its dorsal surface and lateral sides are covered by a thin layer of the neurohypophysis [15, 16]. In the mudskipper, the pituitary was partly, but not completely, embedded in the basal part of the hypothalamus. The adenohypophysis was distinctly divisible into the pars distalis and the pars intermedia. The pars distalis, occupying the anterior portion of the adenohypophysis, is subdivided into the rostal and proximal parts. Specific localization of prolactin cells in the rostral pars distalis and of growth hormone cells in the proximal pars distalis was in agreement with the boservations in other teleosts [17].

Prolactin is well established as a freshwater-adapting hormone in teleosts, primarily restoring plasma sodium levels otherwise lost after hypophysectomy, and activation of prolactin cells has been repeatedly observed when euryhaline species were exposed to fresh water or hypotonic environment [1–3]. Prolactin has also been known to have hypercalcemic action in several teleost species [18, 19]. In this study, significant increases in the nuclear and cell sizes of prolactin cells were observed in the fish in fresh water as compared with those in 100% or 15% seawater. Activation of prolactin cells was well correlated with significant reduction in plasma sodium and calcium levels, indicating prolactin's important roles in freshwater adaptation also in the mudskipper. Lee and Ip [12] reported significant increase in plasma prolactin concentrations, as measured by heterologous radioimmunoassay, in *P. chrysospilos* submerged in waters of low salinities as well as in fish out of water. Heterologous radioimmunoassays have been previously developed and used to measure "relative" plasma prolactin levels in other teleost species such as salmonids, but the validity of the data remains problematic [20, 21]. Development of a homologous radioimmunoassay for mudskipper prolactin is called for to further clarify its mode of actions.

Recent studies have indicated that growth hormone is involved in seawater adaptation of salmonid fish [3–6]. In the present study, there was no change in the nuclear or cell sizes of growth hormone cells after transfer of the mudskipper form 50% seawater to 100% seawater, hypotonic 15% seawater or fresh water. This does not necessarily imply that growth hormone is not involved in osmoregulation of the mudskipper, since changes in the morphology of the adenohypophysial cells may be observed under extreme conditions. Lee and Ip [12] suggested that thyroxine is involved in terrestrial adptation of *P. chrysospilos*. Significant decrease in plasma thyroxine concentrations was observed when the fish were submerged in water of various salinities as compared with the control fish with the liberty to be in or out of 50% seawater, whereas a significant increase was seen in fish kept out of water. Further studies are certainly called for to clarify the role of hormones in their osmoregulatory ability in water as well as in their capability of surviving on land.

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